CONTROL OF ESTRUS AND PROGESTERONE LEVELS IN HEIFERS GIVEN INTRAVAGINAL PROGESTERONE COILS AND INJECTIONS OF PROGESTERONE AND ESTROGEN

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SUMMARY

Fifty-three mature Hereford-cross heifers, which were between day 0 and 3 of the estrous cycle, each had silastic coils containing 2.1 g progesterone inserted into the vagina where they remained for 12 days. Animals were assigned to treatment groups and were injected with (1) 5 mg estradiol benzoate, (2) as (1) + 50 mg progesterone or (3) as (1) + 200 mg progesterone at the time of insertion of the coils. Blood samples were taken for radioimmunoassay of progesterone in plasma before insertion and daily while the coils were in the vagina. There were no differences among treatments in the percentage of heifers observed in estrus from days 1 to 6 after removal of the coils, but a greater percentage of animals in treatments 1 and 3 than in treat 2 were in estrus within 2 days of removal of the coils (P<.05). The mean daily plasma levels of progesterone during treatment for animals on the different treatments, which were in estrus within 2 days removal of the coils were not different. However, for animals in treatment groups 1 and 2 lower average progesterone levels over the first 2 days after insertion appeared to be associated with poor synchronization. The level of progesterone over the last 3 days of treatment was significantly related to the interval from removal of coils to onset of estrus for all heifers (P<.01).

(Key Words: Progesterone, Estrogen, Heifers, Estrus, Silastic Coils.)

INTRODUCTION

Treatment with progesterone or progestogen for less than the length of a normal estrous cycle is effective in synchronizing estrus in cattle and results in normal fertility (Wiltbank and Kasson, 1968; Mauleon, 1974; Roche, 1974; Wishart and Young, 1974), provided the lifespan of the corpus luteum (CL) is shortened. This necessitates the use of a luteolytic agent; consequently if estrogens are used, they are ineffective when given to animals between day 0 and 3 and between day 17 and 20 of the estrous cycle (Wiltbank, 1966; Mauleon, 1974; Roche, 1974). Injecting 50 mg progesterone with the estrogen at the time of insertion of subcutaneous implants containing 4.0 g progesterone resulted in a precise onset of estrus after a 12-day treatment in all animals except those between day 0 and 3 of the cycle at the start of treatment (Roche, 1974). Mauer et al. (1975) have shown that intravaginal silastic coils, of large surface area, increased blood levels of progesterone from < 1 ng/ml to 6 ng/ml within 90 min of insertion and maintained concentrations sufficient to prevent estrus for 21 days. Accordingly, in the trial described here, coils were used to administer progesterone for 12 days to control estrus in heifers. Coils were inserted into the vagina of heifers between day 0 and 3 after estrus. The objective of the experiment was to determine if the variability in the onset of estrus could be reduced by giving an intramuscular injection of progesterone in conjunction with estrogen on the first day of treatment.
day of a 12-day progesterone treatment using intravaginal silastic coils.

MATERIALS AND METHODS

Mature Hereford-cross heifers were penned with vasectomized bulls on pasture and were checked twice daily for the occurrence of estrus which was taken as day 0 of the estrous cycle. Intravaginal silastic coils (30 cm × 3.2 cm × 3 mm when uncoiled, Roche, 1976) containing 6.6% progesterone by weight (2.1 g), were inserted into 56 heifers between day 0 and 3 after estrus; three heifers which lost coils were not used. The heifers were allocated at random in replicates to three groups of 18, 18 and 17 animals, respectively. At the time of insertion of the coils, heifers were given intramuscular injections of corn oil (10 ml) containing either (i) 5 mg estradiol benzoate (ii) 5 mg estradiol benzoate + 50 mg progesterone or (iii) 5 mg estradiol benzoate + 200 mg progesterone. The coils were removed after 12 days and each animal was individually checked for estrus at 830, 1630 and 2030 hr with a vasectomized bull. Heifers not in estrus were penned with another vasectomized bull during the night.

Blood samples were obtained from all animals by jugular venipuncture before insertion of coils and then daily while the coils were in the vagina. Plasma was obtained by centrifugation at 4 C and stored at -20 C until assayed by radioimmunoassay for progesterone as described previously (Gosling et al., 1975). Briefly, plasma samples (2 ml) were extracted with 2.5 ml petroleum ether, 40 to 60 C boiling range. The assay buffer was .1% (w/v) gelatin in .14 M NaCl, .01% NaN3, .01 M NaH2 PO4·NaOH, pH 7.0. The assay volume was .2 ml and contained 1/8000 sheep antiserum to progesterone-11-hemisuccinate-bovine serum albumin and 1, 2, 6, 7-3H progesterone (49,000 dpm, 80 pg; Radiochemical Centre, Amersham). A charcoal separation procedure was used to prepare the bound fraction. Repeated assay of a pool of bovine plasma with each batch of samples gave a mean concentration of 5.63 ng/ml and a coefficient of variation of 13.8% between the means of duplicate determinations. Repeated assay of low level plasma samples plus 500 pg of standard progesterone gave a net recovery of 102.4% (512 ± 14 pg, N = 32).

Samples from 47 animals were selected at random and assayed with similar numbers per treatment, due to other demands on the assay. Differences in estrous response were tested using chi-squares analysis with a single degree of freedom. The mean levels of progesterone the first 2 and the last 3 days of treatment were calculated to minimize extraneous effects. A regression analysis of progesterone levels for these periods on the interval in days between removal of coil and estrus was conducted. Where an animal (9% of total) was not observed in estrus for the duration of the experiment, this interval was taken to be 7 days for the purpose of this analysis.

RESULTS AND DISCUSSION

Estrous Response. There were no differences among groups in the proportions of heifers observed in estrus within 6 days following removal of the coils (table 1). However, there were significant differences in the temporal pattern of the onset of estrus. More heifers were in estrus within 2 days of removal of the coils in groups 1 and 3 than in group 2 (P<.05).

Progesterone Levels. It can be seen from figure 1 that the mean daily plasma levels of progesterone during treatment for animals in estrus within 2 days of removal of the coils were similar among groups. Progesterone levels in figure 2 for animals not detected in estrus or

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<tr>
<td>In estrus .... No.</td>
<td>17</td>
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<sup>a</sup>The occurrence of estrus is recorded relative to the day of coil removal (Day 0).
in estrus more than 2 days after removal of the coil are different from levels in figure 1. Higher levels of progesterone at the end of treatment were associated with a longer interval to estrus (figure 1 vs figure 2) and the pooled regression of progesterone levels at the end of treatment

Figure 1. Mean (± standard error) daily plasma levels of progesterone while the coils were in the vagina for animals detected in estrus within 2 days after removal of the coils. T is the blood level of progesterone just before treatment. N is the number of animals per treatment. Group 1. 5 mg estradiol benzoate. Group 2. 5 mg estradiol benzoate + 50 mg progesterone. Group 3. 5 mg estradiol benzoate + 200 mg progesterone.

Figure 2. Mean (± standard error) daily plasma levels of progesterone while the coils were in the vagina for animals detected in estrus more than 2 days after removal of the coils or not detected in estrus. T is the blood level of progesterone just before treatment. N is the number of animals per treatment. Group 1. 5 mg estradiol benzoate. Group 2. 5 mg estradiol benzoate + 50 mg progesterone. Group 3. 5 mg estradiol benzoate + 200 mg progesterone.
on interval to estrus was significant ($P<.01$) with no evidence for heterogeneity among treatment groups. The slope of the pooled regression line was .266.

The levels of progesterone at the beginning of treatment affected the subsequent interval to estrus after treatment. The pooled regression of progesterone levels at the beginning of treatment on the subsequent interval from coil removal to estrus was not significant but there was some evidence for heterogeneity among treatment groups ($P<.01$). For groups 1 and 2, the regression coefficients were $-.606$ and $-.513$, respectively, ($P<.05$) whereas for treatment 3 it was .164.

Following short-term progesterone treatments in cattle, the interval to estrus in heifers is influenced by the stage of the estrous cycle at the start of treatment, animals between day 0 and 3 being most variable in response (Roche, 1974). In such animals, injection of estrogen or estrogen with two dosage levels of progesterone on the first day of 12-day progesterone regime with silastic coils resulted in similar numbers showing estrus after coil removal but did affect the pattern of onset of estrus. Significantly ($P<.05$) more heifers were in estrus on day 2 after removal of the silastic coils in the groups of heifers receiving 5 mg estradiol benzoate alone or with 200 mg progesterone at the start of treatment. The fact that estradiol benzoate alone was effective in this experiment is in contrast to earlier research (Mauleon, 1974; Roche, 1974; Woody and Pierce, 1974; Wiltbank and Gonzalez-Padilla, 1975) and may be due to the initial rapid increase in progesterone from the coil (Mauer et al., 1975). However, there is no obvious explanation for the greater spread in the onset of estrus after injection of 50 mg progesterone + 5 mg estradiol benzoate at the beginning of the 12-day treatment (group 2).

The levels of progesterone during treatment in all animals observed in estrus within 2 days of removal of the coils were similar irrespective of treatment used and consisted of high levels the day after insertion which declined steadily to day 7 or 8, and then more slowly to the end of treatment. However, for animals given none or 50 mg of progesterone combined with the estrogen injection, a lower level of progesterone at the beginning of treatment was associated with a longer interval from removal of the coil to estrus. The relationships between these lower progesterone levels at this time and subsequent control of CL function are not clear, since such differences were not found in similar animals receiving 200 mg progesterone. A longer interval to estrus in animals from all treatment groups was associated with higher levels of progesterone over the last 3 days prior to the end of treatment, indicating incomplete inhibition of CL function, suggesting that both endogenous and exogenous progesterone were being measured. Determination of blood levels in ovariectomized heifers would allow better interpretation of the contribution of progesterone from the coil and from the CL.

**LITERATURE CITED**


